EPL BIO ANALYTICAL SERVICES

Validation of an Automated Procedure for the Determination of Total Dietary Fiber in Grains and Meals

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Abstract

Dietary fiber is a mixture of complex organic substances and was initially defined as the remnants of plant cells resistant to the alimentary enzymes of humans. The definition has been modified to include hemicelluloses, celluloses, lignins, pectins, gums, nondigestible oligosaccharides and waxes. The current definition includes soluble and insoluble forms of dietary fiber. The purpose of this study was to validate an analytical procedure for the determination of total dietary fiber (TDF) in maize grain, soybean seed, soybean meal, cotton seed, and canola seed using a commercial automated TDF analyzer[1]. TDF and other nutritional and compositional data for these matrices are required by global regulatory agencies to support registration of genetically modified seed. Automated procedures which reduce labor input and total time required to complete the assays are an important factor in meeting regulatory data submission deadlines and increasing laboratory capacity. The automated procedure is based on AOAC official method 991.43[2]. Manual procedures specified in AOAC 991.43 for pre-extraction of fat, multiple enzyme treatments to remove protein and starch, and multiple filtration steps are time consuming, labor intensive and tedious. The automated procedure significantly reduces the labor input and filtration time for many of these steps. Validation parameters included determination of measurement precision by calculation of the relative standard deviation (RSD) and comparison of TDF values obtained using the automated procedure with values obtained using AOAC 991.43. Measurement precision for the automated procedure was superior to the precision reported for AOAC 991.43 for all matrices tested. In addition, the automated procedure produced values that were statistically comparable to the historical range established in our laboratory using AOAC 991.43. The automated procedure was successfully validated and is currently utilized for routine analysis of grains and meals.

Objective

To validate an automated procedure for the determination of total dietary fiber in grains and meals.

Chemicals and Reagents

- 95% Ethanol, technical grade, EMD, Darmstadt, Germany
- Acetone, ACS, EMD, Darmstadt, Germany
- Heat-stable α-Amylase, Megazyme, Wicklow, Ireland
- Protease, Megazyme, Wicklow, Ireland
- Amyloglucosidase (AMG), Megazyme, Wicklow, Ireland
- Sodium Hydroxide (NaOH), 6N, Fisher Scientific, St. Louis, MO
- Hydrochloric Acid (HCl), 6N, Fisher Scientific, St. Louis, MO
- MES [2-(N-Morpholino)ethanesulfonic acid] 0.05M, Sigma-Aldrich, St. Louis, MO
- TRIS [Tris(hydroxymethyl)aminomethane] 0.05M, Sigma-Aldrich, St. Louis, MO
- Celite C-211, Acid-washed, Ankom, Macedon, NY
- pH buffer solutions, concentrations of 4, 7, and 10, Poole Dorset, United Kingdom
- Deionized (DI) Water

Test Matrices

The method was validated using maize grain (*Zea mays*), soybean seed (*Glycine max*), soybean meal, cotton seed (*Delta opal*), and canola seed (*Brassica napus*).

Equipment

- Dietary Fiber Instrument, Ankom ™ Dietary Fiber Analyzer, Ankom, Macedon, NY (Figure 1.)
- Protein Analyzer, Kjeltec™ 8400, Foss, Hillerød, Denmark
- Muffle Furnace, Fisher Scientific, St. Louis, MO
- Forced Air Oven, Blue M Electric Company, Blue Island, IL
- Balances, 0.1 and 1 mg accuracy, Mettler Toledo, Columbus, OH
- pH Meter, Thermo Scientific, Pittsburgh, PA
- Centrifuge, Eppendorf, Hamburg, Germany
- Orbital Shaker, New Brunswick Scientific, Enfield, CT
- Vortex Apparatus, VWR, Radnor, PA
- Desiccator and Desiccant pouches, Dri-Rite, Chicago, IL
- Pipetters, adjustable and fixed volume, Thermo Scientific, Pittsburgh, PA
- Bottle top Dispenser or Repeater Pipette, Thermo Scientific, Pittsburgh, PA

Equipment

- Soluble Dietary Fiber (SDF) filter bags, Ankom, Macedon, NY
- Insoluble Dietary Fiber (IDF) Flow-Thru bags, Ankom, Macedon, NY
- Drying Rack, Ankom, Macedon, NY
- Rinse Stand, Ankom, Macedon, NY
- Heat Sealer, Ankom, Macedon, NY
- Bag weigh holder, Ankom, Macedon, NY
- Class A Volumetric Glassware, Chemglass, Vineland, NJ
- Ceramic Crucibles with lids, Online Science Mall, Pinson, AL
- Weigh Paper/weigh boats/aluminum pans, VWR, Radnor, PA
- 50 mL Centrifuge tubes, VWR, Radnor, PA
- Thermometer, Nova-Tech International, Kingwood, TX
- Retsch centrifugal grinder with 0.75 mm screen, Hahn, Germany
- Scissors
- Wash Bottle

Figure 1. Dietary Fiber Instrument, Ankom ™ Dietary Fiber Analyzer[1]

Procedures

- 1.25 mL of α-Amylase, 2.5 mL of Protease, and 5.0 mL of AMG were diluted to 25 mL in 25 mL volumetric flasks. All reagents were added to designated containers on instrument.
- Crucibles were conditioned for 3 hours in a muffle furnace with a temperature set at least 600° C. Cooled and weighed crucibles were stored in desiccators.
- Samples were ground with dry ice using a Retsch centrifugal grinder equipped with a 0.75 mm screen. Samples were stored frozen at -20° C. All samples are weighed and analyzed in duplicate for protein and ash calculations. Celite (1.0 g) was weighed into aluminum pans. Sub-samples of soy meal and maize grain (0.5 g) were weighed into aluminum pans. Sub-samples of soy seed, cotton seed, and canola seed (0.5 g) were weighed into 50 mL centrifuge tube for defatting.
- 25 mL of acetone was added to each centrifuge tube and tubes were shaken on an orbital shaker for at least 2 hours. Samples were removed from shaker and centrifuged at 300 rpm for 10 minutes. Acetone layer was decanted and samples were left in hood to evaporate overnight.

Procedures

- SDF filter bags and IDF Flow-Thru bags were installed on Ankom™ Dietary Fiber Analyzer. Celite and sample were rinsed with an automatic pipette into clamped SDF bags and IDF bags, respectively, using 3 mL of DI water. Bags were sealed shut using clamps bars. "AOAC 991.43 Function" and "TDF Procedure" were selected on instrument. Instrument heated and agitated sample bags during automatic addition of reagents throughout extraction. pH was manually adjusted prior to addition of AMG to ensure a pH between 4.3 and 4.9. Bags were filtered and rinsed by instrument after extraction. SDF bags were removed and placed on a rinse stand after instrumental analysis was completed.
- Bags were rinsed three times with 10 mL of acetone using a spray bottle. Each bag was sealed directly above the filter using a heat sealer after acetone was allowed to evaporate for 15 minutes. Bags were dried in an oven set at 105° C ± 3° C for 90 minutes then placed in desiccant pouches and weighed once cool.
- One replicate of sample was analyzed for Protein using Kjeltec™ 8400 and one replicate was ashed in conditioned crucibles in an oven with a temperature of least 600° C for 3 hours.

Calculations:

Sample Weight DB (g) = Sample Weight (g) x % Dry Matter ÷ 100
DB is Dry Basis.
% Dry Matter is obtained from moisture determination.

Residue Weight (g)
= Dried Filter Bag and Residue Weight (g) – Filter Bag Weight (g)
– Celite Weight (g)

Protein (g) = Residue for Protein Analysis (g) x Instrumental Protein Results ÷ 100

Ash (g) = Ashed Crucible, Bag, Residue Weight (g) – Empty Crucible Weight (g)
– Celite Weight (g)

Blank (g) = (Average R_{B1} *and* R_{B2}*) –* P_{Blank} *–* A_{Blank}
Negative blanks are not used in the %TDF calculation.

$$TDF\,(\%DB) = \frac{(Average_{F1}\ and\ Average_{F2}) - P - A - B}{Average\,Sample\,Weight\,DB\,(g)}\,x100$$

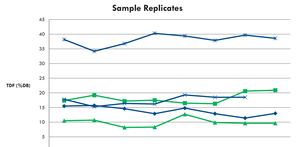
Where: R_{B1}, R_{B2} = Residue weight for duplicate blanks (g)
R_1, R_2 = Residue weight for duplicate samples (g)
P = Protein of residue (g)
A = Ash of residue and bag (g)
B = Blank (g)

Results/Discussion

The time necessary to complete TDF analysis using AOAC method 991.43 is three days. The automated TDF analyzer reduces that time to two days. Filtering of 16 samples is reduced from 8 hours to 1 hour. Protein and ash methods remain the same. Automation allows the analyst to perform and prepare for other stages of analysis.

Eight replicate sub-samples (Table 1) of each matrix (except canola seed where 7 replicates were used) were analyzed to determine the accuracy and precision of the developed method in comparison with values obtained using AOAC 991.43. Method performance requirements included a maximum Relative Standard Deviation (RSD) (n=8) of 15% (Table 2.) and that each replicate determination for the automated method must fall within three standard deviations (±3SD) of the mean result obtained using method 991.43. Good agreement between validation data and literature values were observed (Table 3).

Table 1. TDF (%DB) values for each sample analyzed



Soy Seed	13.5	13.7	14.6	12.9	14.8	12.9	11.4	13.0
Soy Meal	17.4	19.2	17.2	17.5	16.5	16.3	20.6	20.9
Maize Grain	10.5	10.7	8.22	8.32	12.7	9.88	9.64	9.64
Canola Seed	17.8	13.3	16.4	16.2	19.3	18.5	18.5	
Cottonseed	38.2	34.2	36.8	40.3	39.4	37.9	39.7	38.6

Table 2. Mean, Standard Deviation, and Relative Standard Deviation (RSD) for all matrices.

Matrix	TDF (%DB) Mean	Std. Dev. (n≥7)	RSD (%)
Soy Seed (n=8)	13.8	1.52	11.0
Soy Meal (n=8)	18.8	1.80	9.91
Maize Grain (n=8)	9.95	1.43	14.3
Canola Seed (n=7)	17.4	1.48	8.48
Cottonseed (n=8)	38.1	1.93	5.06

Table 3. Comparison of TDF values and Literature values.

Matrix	Automated TDF Range (%DB)	Literature Range (%DB)	AOAC 991.43 Mean – 3SD (%DB)	AOAC 991.43 Mean +3SD (%DB)	AOAC 991.43 Mean (%DB)
Soy Seed	11.4-15.7	10.2-20.3[3]	N/A	N/A	N/A
Maize Grain	8.22-12.7	11.1-15.6[4]	7.9	18.5	13.2
Soy Meal	16.3-20.9	N/A	N/A	N/A	N/A
Canola Seed	15.3-19.3	11.0-12.5[5]	13.3	46.7	30.0
Cottonseed	34.2-40.3	33.69-47.55[4]	36.2	53.6	44.9

Conclusions

A rapid automated analytical method for the determination of total dietary fiber in seeds and meals was successfully validated. The automated method demonstrated acceptable and produced TDF values comparable to historical values obtained using AOAC 991.43 for matrices which data for comparison was available (Table 3).

Acknowledgements

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References

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(2) AOAC International Method 991.43 In Official Methods of Analysis of AOAC International, 17th Edition. Association of Official Analytical Chemists International, Gaithersburg, Maryland.

(3) NFRI-NARO (National Food Research Institute-NARO) (2011-up to 2009 harvest data), "Soybean" *Food Composition Database for Safety Assessment of Genetically Modified Crops as Foods and Feeds*, Japan

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